Gene Action in Relation to Growth and Development. I. Phenotypic Variability

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GENE ACTION IN RELATION TO GROWTH AND DEVELOPMENT. I. PHENOTYPIC VARIABILITY

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Among the most fundamental problems in biology belongs the action of the gene. The gene, however, is elusive since no direct approach is possible: the gene can only be recognized by its effect. These effects may be used either in their final expression—the heritable characters—and then we are in the domain of classical genetics. Or we can try to infer from the final and intermediate steps of gene action what lies at the beginning of this chain. This is the approach of both the biochemist, the embryologist, and the physiologist. The successes of the biochemist in approaching the action of the gene are spectacular, such as in the analysis of biosynthesis in Neurospora.

The physiologist or embryologist cannot hope to contribute much in the direction of the chemistry of gene action, since they are dealing with processes rather than specific biochemical reactions. But they can contribute in several other directions. On the one hand they are dealing with growth and development, which cannot be expressed in chemical terms as yet but which are of such importance in inheritance. On the other hand they stress the quantitative side of biological processes more than the biochemist, who, in his analysis of the action of the gene, largely stresses the pathways of gene action.

The following discussion will deal primarily with physiological observations which have a bearing on the quantitative side of gene action. This problem has many different aspects. The problem of practically absolute uniformity in gene reproduction will not be dealt with.

In gene action, we find absolute reproducibility in ontogeny, in which the sequence of events and of organs meets only very seldom with a slight deviation in timing, but the number and order of, for example, flower parts in the pea flower are practically without variability.

Not only the qualitative aspects, but also the quantitative side of growth is genetically controlled. Here we meet with a much greater variability: Even within a pure line or clone there is considerable variation in size or weight or any other quantitative character.[1] Two factors contributing to this phenotypic variability have been recognized. In the first place, the environment has a great effect on the expression of quantitative characters. Hormonal control of growth insures proportionality between the different parts of the organism: when for some reason one part is small, most other organs are proportionately reduced in size. The absolute di-

mensions of the organism are therefore less directly gene-controlled. The proportions, however, are under gene control. Yet the size of an organism under a specific set of growing conditions, and particularly its maximal size, is definitely an inheritable character.

In the second place, all cellular reactions occur on a microscopic scale, where the molecular structure of matter starts to interfere with the regularity of classical statistical physics. This has been pointed out by many physicists,[2-4] and was very clearly expressed by P. Jordan in 1936: "Wide applicability of macrophysical laws to organisms is unquestioned; yet there are good grounds to believe that the basic life processes do not belong in the realm of macrophysics any more. The macroscopical processes occurring in the larger parts of the body are governed by considerably more delicate processes. The ultimately controlling reactions are generally of atomic dimension." This leads Jordan to his "amplifier" theory of organisms, in which he stresses the fact that in organisms the basic controlling "reactions of atomic dimensions" such as gene reactions, are amplified to macroscopic observability by cellular processes. "Genetics provides the most convincing and widest basis for the thesis, that organisms are not macrophysical, but microphysical systems."

Continuing his argument, Jordan then states that unstability, or inconstancy, is a fundamental characteristic of quantum physics and that actually inconstancy occurs in biology as well, as the statistical nature of the Mendelian laws shows. From this, one might conclude that when genes control development, one would expect a fundamental variability in organisms. This thesis was actually elaborated by Otto Rahn[5] who calculated from the observed variability in biological material (in his case bacteria) the numbers of genes involved. He obtained figures ranging from 100 to 1000, which agree with estimates made of the number of genes occurring in organisms using other premises.

Probably few other biologists rationalized their acceptance of phenotypic variability as due to control of growth and development by atomistic reactions in the sense of Jordan (see, e.g., the criticism of Bünning).[6] Yet we find that not only geneticists, soil scientists and agriculturists, who have to work with variable material of which they cannot reduce the variability, but also biologists, have adopted statistical analysis as the general method of coping with variability in biological material. This indicates to what extent phenotypic variability was accepted as a basic property of living matter by biologists as a whole. If phenotypic variability had been considered as being largely due to environment, more serious efforts would have been made to control the external environment of growing plants.

Experience gained in the Earhart Plant Research Laboratory shows that environment rather than the atomistic nature of biological reactions is responsible for phenotypic variability. It was generally observed that plant

material grown under the controlled conditions of this laboratory was less variable than similar material grown in an ordinary greenhouse. This was of practical importance in the design of experiments, in which fewer objects could be used to obtain the same significance, even to the extent that only four plants are now used per treatment in many experiments. Justification for this reduction in size of sample in experiments is found in figure 1. This shows the effects of an increase in uniformity of growing

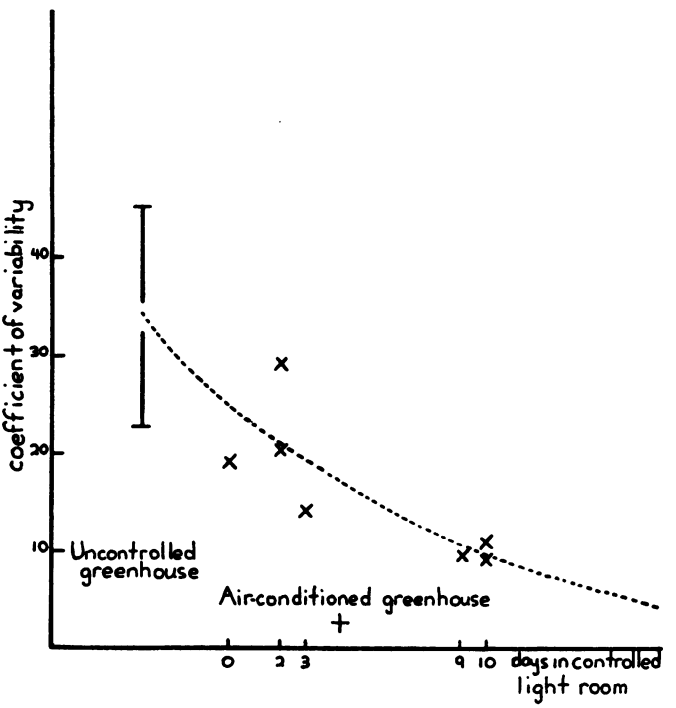

FIGURE 1

Coefficient of variability of dry weight of tomato plants grown in either an ordinary greenhouse (*left*) or in an air-conditioned greenhouse (*right*). The latter plants were kept for 2, 3, 9, or 10 days (abscissa), prior to weighing, in controlled light rooms with even more uniform environment.

conditions on variability in weight of young tomato plants, when they are grown with different degrees of environmental control. In most plant material we find that the coefficient of variability—when the standard deviation of the mean is expressed in per cent of the average—amounts to anywhere between 20 and 40 per cent. In the Earhart greenhouses this variability is reduced, usually to about 15 per cent. Yet, when similar plants are grown in temperature- *and* light-controlled rooms, their coef-

ficient of variability is decreased to about 8 per cent. The details of these determinations will be published later.

At present, the question cannot be answered how much of the remaining 8 per cent variability is innate, and how much is imposed by inconstancy of growing conditions. In the following observations the coefficient of variability in pea plants decreases to below 4 per cent (table 1) and it is very likely that further work will reduce this still more.

We can view this problem of variability from many other angles. If growth were controlled by processes whose rates are subject to appreciable random fluctuations, the variability of a batch of plants should remain the same or perhaps even increase as they continue to grow. This is not actually the case as field observations will show: Even though germination may be variable in a wheat or corn field and the seedling stand may consist of fairly uneven plants, a well-tended field of mature wheat or corn is

TABLE 1

LENGTH IN MM. OF PEA PLANTS, GROWN IN A 16-HR. PHOTOPERIOD AT 17°C. IN DIFFERENT INTENSITIES. EACH GROUP CONSISTED OF 9 PLANTS; IN EACH CASE THE STANDARD ERROR OF THE MEAN IS GIVEN AND THE COEFFICIENT OF VARIABILITY

	VINCO, 1500 FT.-C.		VINCO, 1000 FT.-C.		VINCO, 500 FT.-C.		KRONBERG, 1500 FT.-C.	
DATE	LENGTH	COEFF. OF VARIA.	LENGTH	COEFF. OF VARIA.	LENGTH	COEFF. OF VARIA.	LENGTH	COEFF. OF VARIA.
23 VII	34.3 ± 1.4	11.5	35.8 ± 1.3	10.3	37.5 ± 1.5	11.3	32.4 ± 2.0	17.4
25 VII	51.8 ± 2.0	10.9	49.3 ± 2.0	11.5	53.3 ± 2.3	12.2	45.9 ± 2.7	16.5
31 VII	107.2 ± 2.6	6.8	107.8 ± 2.2	5.9	104.2 ± 2.7	7.3	80.9 ± 5.2	18.1
7 VIII	189.4 ± 3.0	4.5	191.3 ± 3.1	4.8	163.5 ± 4.1	7.1	142.4 ± 7.7	15.3
12 VIII	235.0 ± 3.0	3.6	248.9 ± 4.0	4.6	205.0 ± 3.9	5.4	.....	..
13 VIII	249.6 ± 3.0	3.4	268.1 ± 4.0	4.2	223.5 ± 4.2	5.3	194.9 ± 6.3	9.1
Dry wt. in mg.	870 ± 23	5.2	857 ± 37	12.1	415 ± 9	6.1	762 ± 53	20.0
Actual increase in length from 31 VII to 13 VIII	142.3 ± 2.6	..	.....	..	.....	..	114.0 ± 3.6	..

surprisingly uniform. The following set of measurements substantiates this field observation. Groups of "Vinco" and "Kronberg" peas were grown in three different light intensities: 500, 1000, and 1500 ft.-c., and as they grew, the distance to the artificial lights was adjusted so that their tops continued to receive the same intensities.

To start with, groups of nine plants were selected to be as uniform as possible in respect to size and form. They were measured from time to time, and table 1 and figure 2 show the results. The variability was small to start with in all three groups; in absolute figures the variability increased least in the peas growing in the highest light intensity. But the relative variability decreased very much—to less than one-half of what it originally was. Because the variability in these peas was so small to start with, the causes for its decrease are not so clear. In the

Kronberg variety which was more variable at the start, variability remains much greater, but not because the longest plants continue to grow fastest.

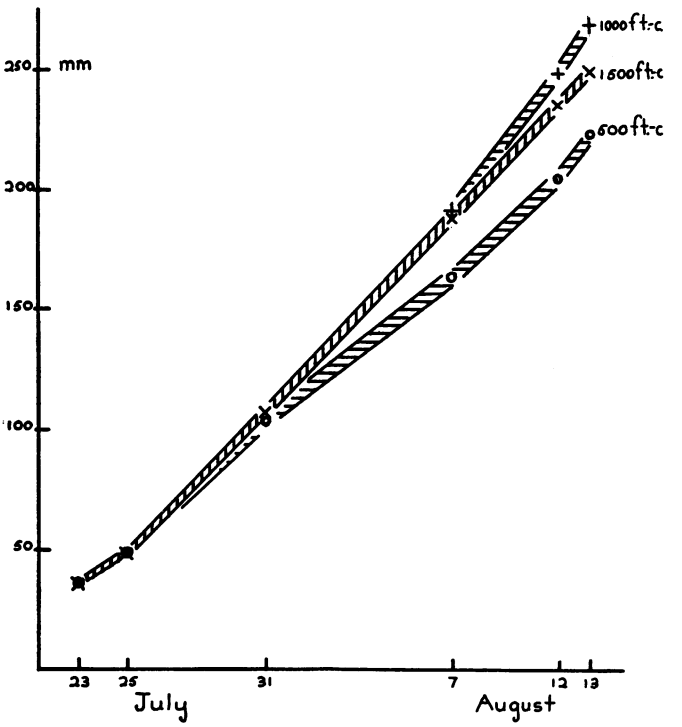

FIGURE 2

Length of Vinco pea plants grown in a 16-hour photoperiod at 17°C. in different light intensities. Variability of each group of plants is indicated by a 2 × standard error.

For it is seen that the variability of the length increase during the last fourteen days is less than the previously existing variability (the largest

TABLE 2

GROWTH OF PEA PLANTS DEVELOPING FROM SEEDS OF DIFFERENT WEIGHT. AVERAGE OF 25–35 PLANTS PER GROUP

WEIGHT OF DRY SEED, MG. AVERAGE	WEIGHT OF DRY SEED, MG. RANGE	GROWTH IN MM. AUG. 13–18	GROWTH IN MM. AUG. 18–21	GROWTH IN MM. AUG. 21–26	TOTAL LENGTH, AUG. 26
180	130–199	24.2	36.5	73.5	134.2
230	201–246	22.2	37.5	77.7	137.5
330	298–377	20.7	31.2	84.0	136.0

differences on July 31 were 54 mm.; on August 13, 69 mm., whereas for the length increments they were only 34 mm.).

These measurements show that the growth rate of these peas does not depend on the preceding conditions, and that differences within a well-selected strain are neither genetically nor phenotypically conditioned. Differences between individuals do not become magnified, but decrease relatively. Therefore we have to consider the possibility of a self-regulatory mechanism which adjusts the growth rate of these plants. This also follows from the observation that originally the plants from bigger pea seeds grow slower than those from smaller ones; after some time they catch up with the plants from the smaller seeds and reach the same overall size, as shown in table 2. Similar results were obtained when plants were arranged according to their size and growth rate after one week's growth. Those growing fastest reduce their growth rate in the following eleven days to below that of the originally slower growing peas to such an extent that their final size was within 0.7 per cent of each other, whereas originally this group was 17 per cent larger (see Fig. 3). Even the very smallest peas (only 45 per cent of the largest) caught up with the others.

TABLE 3

COEFFICIENT OF VARIABILITY OF TOMATO PLANTS WHEN GROWN IN SUBOPTIMAL, OPTIMAL AND SATURATING LIGHT INTENSITIES AT 17°C. IN A 16-HR. PHOTOPERIOD. THE VARIABILITY IS BASED ON THE DRY WEIGHTS OF 20 CONTAINERS WITH 20 PLANTS EACH, AS IN FIGURE 1

LIGHT INTENSITIES, FT.-C.	COEFFICIENT OF VARIABILITY	AVERAGE, %
500–700	12.0; 12.5; 14.2	12.9
900	8.4; 8.9; 8.9	8.7
1100–1500	9.8; 10.2; 10.7; 11.6; 12.0; 13.3	11.3

This was especially evident when a correction was made for the physiological age of the small plants—which is about two days less than that of the other peas—then their growth rate fell within the range of the originally taller plants.

In connection with variability of experimental material another observation has to be recorded. Under optimal growing conditions, when rate of elongation is greatest, the coefficient of variability is least (see Fig. 4). This was found in young pea plants grown at different temperatures. It is interesting to note that in the youngest stage, the growth rate is unaffected by temperature over the 15.5° to 26°C. range. Only below 15° is it controlled by a process with a high temperature coefficient. And it is just where growth is presumably limited by a chemical process that variability increases.

In tomato experiments it was also found that under most nearly optimal growing conditions the variability of the plants was the least; therefore, also at the greatest rates of growth. For instance, the average dry weights of tomato plants grown like those in figure 1 showed a coefficient of varia-

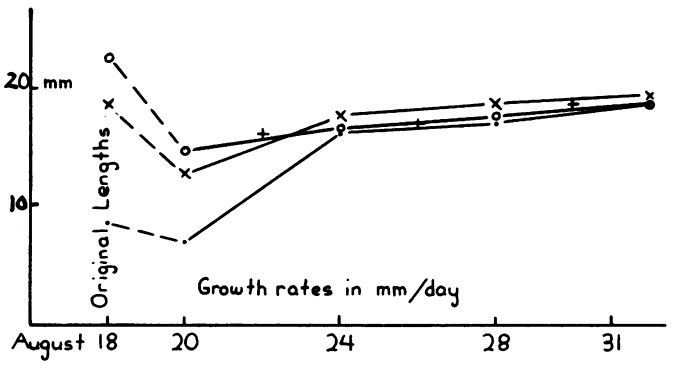

FIGURE 3

Growth rates of Vinco peas in mm./day (ordinate). Plants were divided into 3 groups: the shortest, tallest, and intermediate, and the growth of each group was followed for 2 weeks.

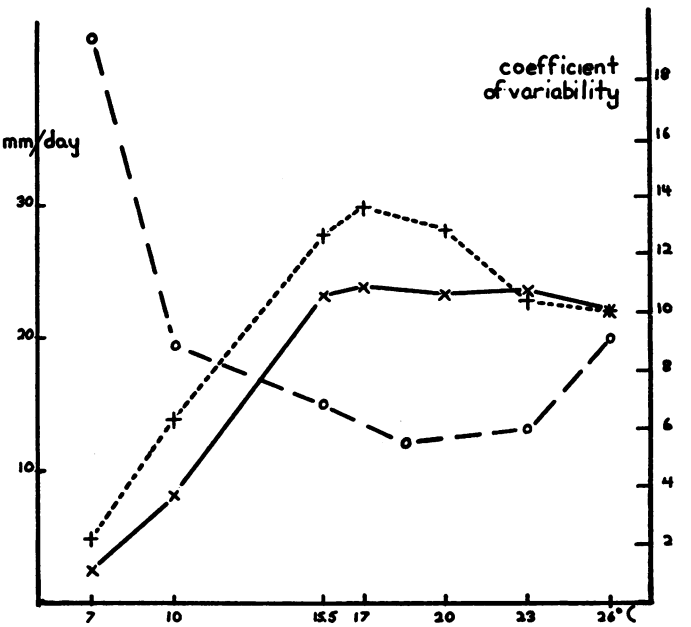

FIGURE 4

Growth rates (left ordinate, mm./day) of Vinco peas at different temperatures (abscissa) for the period of 5–13 days after planting (crosses and solid line) and 13–20 days after planting (plusses and dotted line). Coefficient of variability (right ordinate) of the growth rate from 5 to 13 days shown in circles with broken line.

bility of 12.9 per cent when grown at suboptimal light intensity, and of 11.3 per cent at supraoptimal intensities, but of 8.7 per cent at the optimal intensity (table 3).

We can draw a number of conclusions from these observations:

(1) Variability in the material described is smaller than ordinarily supposed to be typical for genetically uniform plants. The selected examples are not exceptional, however, and variability of plants in the Earhart Laboratory is generally much less than when similar plants are grown under uncontrolled conditions. This is due to two independent effects: (*a*) through proper air distribution in the greenhouses and controlled light rooms, the individual plants are more nearly all under the same growing conditions, which they are not, e.g., on an ordinary greenhouse bench where the plants in the center have much less air circulation and therefore are subjected to higher temperatures during the day, and (*b*) under optimal growing conditions variability is also smaller (Fig. 4); with the great variety of growing conditions in the Earhart Laboratory plants in general can be grown under more nearly optimal conditions and consequently will be less variable.

(2) From figure 1 it can be seen that in genetically uniform material, the upper limit of any innate variability is around 5 per cent. The additional variability usually observed is imposed largely by uncontrolled growing conditions. There are two explanations for this low variability.

(3) On the one hand, it is possible that low variability is an innate characteristic of biological material. This seems to be the view of both Bohr and Schrödinger. Bohr[2] states: "The explanation of the properties of living organisms certainly lies in their specific organization, in which typical atomic traits are interwoven with mechanistical traits to an extent which has no counterpart in inorganic nature."

(4) On the other hand, we could assume that growth is variable like any physicochemical process, but that superimposed on it is an autoregulatory process, which corrects any deviation imposed on it by chance. This would seem to be substantiated by the data of figures 2 and 3, from which it follows that the originally slower growing plants grow faster afterward, but in later development this was less pronounced: It took the peas (shown in Fig. 3) which were growing slowest from August 22 to 26 three days to catch up with the fastest growing ones and even then they did not surpass them (originally 23.8 and 27.4 mm./day; next three-day period, 24.0 and 28.7 mm./day; last four-and-a-half days, 28.7 and 28.7 mm./day). Therefore, although there is a tendency for growth of the slower growing plants to pick up, there is no *immediate* response in all cases, and the self-regulatory mechanism is not very intimately tied up with the growth process itself.

(5) Is it possible to imagine a process which controls growth which

could explain the phenomena described in this paper? From figure 4 we can conclude that at the optimal growth rate a diffusion process, largely independent of temperature, controls growth in young peas. Along a very different line of reasoning,[7] it was concluded that diffusion within a cell between the nucleus and cytoplasm is the limiting process in growth.

(6) Such a diffusion process is slower when the cells or organs are larger. Thus when faster growth has resulted in larger cells in the growing point, this would automatically reduce the growth rate. This is just the opposite of control by chemicals, where a larger original structure would tend to increase the supply of hormones or other growth factors and would result in faster growth. We would expect, then, the larger individuals to be both the result of and to result in a greater growth factor supply. Therefore through chemical control of the growth rate, original small differences in size would tend to become magnified, whereas just the opposite occurs (Figs. 2 and 3 and table 2). By the simple assumption that growth is controlled by an intra- or intercellular diffusion process, we can explain a decrease in growth rate of the growing points which have become larger than the average and vice versa.

(7) How can we tie the observed phenomena in with genes? The gene theory was based upon the discontinuity of the hereditary process. The distribution of characters over the offspring is a typical quantized phenomenon, explained by the presence in cells of a few discrete units or genes. The same genic concept can account for effects of ionizing radiation on organisms (see, e.g., Timofeeff-Ressovsky and Zimmer[8]); through these radiation effects the approximate size of the gene can even be calculated.

There is, of course, no doubt that growth and the growth rate in plants is dependent upon the hereditary mechanism. For each pea variety, although very uniform within itself, differs from most other varieties in its growth rate. Yet, since the growth rate is so remarkably uniform from plant to plant, we must conclude that either the growth rate is only very indirectly dependent upon genes, or that growth is controlled by hereditary units which are present, not in twofold, but in very large numbers within each cell.

The former possibility resolves itself into two mechanisms: Either the laws of quantum mechanics do not hold for the reproduction and physiological effects of genes, as Schrödinger[4] suggests, or the gene becomes multiplied in each cell before it exerts its effect. In practice, the latter mechanism becomes almost identical with the assumption of polygenes controlling quantitative processes inside the cell. But whereas formerly the nature of polygenes could only be inferred from the distribution of the quantitative characters over the offspring, it should now become possible to study this problem by the quantitative effect of the hereditary mechanism upon the growth process within an individual. In subsequent papers this will be analyzed in more detail.

The physiologist and embryologist are now in the uncomfortable position where they will have to explain why genes, which were just proved by the geneticist and biophysicist to be following quantum mechanics, do not impart the degree of variability upon growth and development which one would expect from quantum mechanics. We can view this difficulty also from another standpoint, however. If the uncertainty principle of quantum mechanics did hold for gene reproduction and gene effect, organic life in its present form would be impossible, because there would be only statistical reproducibility of organisms, and very soon life would have reverted to the more stable and probable inorganic state. Only because development does not follow the laws of quantum mechanics can the less probable condition of life be continued indefinitely. The reduced variability of genetically uniform plant material when grown under controlled conditions is therefore nothing exceptional; it is the logical consequence of the rigid laws of organic development which fall outside the laws of statistical or quantum mechanics. This has been expressed before by Schrödinger: "The unfolding of events in the life cycle of an organism exhibits an admirable regularity and orderliness unrivaled by anything we meet in inanimate matter."

Summary.—The major part of the commonly encountered variability of genetically uniform material (phenotypic variability) is not due to statistical fluctuations of the numbers of molecules on which development depends, but is largely caused by inconstancy of and irregularities in the external environment. The genetic homogeneity of pure lines can be translated under completely controlled conditions into phenotypic uniformity of plant material which exceeds anything observed thus far. The more optimal the growing conditions are, the smaller is phenotypic variability. When the standard deviation is expressed as per cent of the mean, variability in peas and tomatoes is regularly reduced to 2 per cent, and in the most favorable material it was as little as 1.2 per cent. Due to an autoregulatory mechanism phenotypic variability decreases in the course of development. All these facts lead to the conclusion that the laws of organic development fall outside the laws of statistical or quantum mechanics.

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